Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/GB04/005185

International filing date: 13 December 2004 (13.12.2004)

Document type: Certified copy of priority document

Document details: Country/Office: GB
Number: 0328870.1
Filing date: 12 December 2003 (12.12.2003)

Date of receipt at the International Bureau: 02 May 2005 (02.05.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

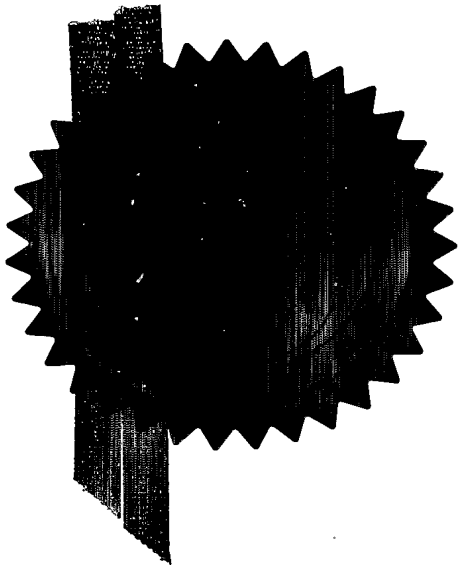
I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

I also certify that the application is now proceeding in the name as identified herein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.



Signed

Dated 20 April 2005

GB0328870.1

By virtue of a direction given under Section 30 of the Patents Act 1977, the application is proceeding in the name of

TISSUOMICS LIMITED,
Lakin Rose,
Enterprise House,
Vision Park,
Histon,
CAMBRIDGE,
CB4 9ZR,
United Kingdom

Incorporated in the United Kingdom

[ADP No. 08984650002]

Pat[illegible]s Form 1/77

Patents Act 1977
(Rule 16)

The Patent Office

15DEC03 E659[illegible]4-4/010006
P01/7700 0.00-03/2870.1 ACCOUNT CHA

1/77

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference — SJC/P/13550.7GB

2. Patent application number
(The Patent Office will fill in this part) — 0328870.1

SNI
12 DEC 2003
LONDON

3. Full name, address and postcode of the applicant or of each applicant *(underline all surnames)* — City University, London
Northampton Square
London EC1V 0HB
United Kingdom

SECTION 30 (1977 ACT) APPLICATION FILED
28/02/03

Patents ADP number *(if you know it)* — 8772253001

If the applicant is a corporate body, give the country/state of its incorporation

12 DEC 2003

4. **Title of the invention** — CHARACTERISING BODY TISSUE

5. Name of your agent *(if you have one)* — Olswang

"Address for service" in the United Kingdom to which all correspondence should be sent

(including the postcode) — 90 High Holborn
London WC1V 6XX

Patents ADP number *(if you know it)* — 7627995002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* each application number

Country	Priority Application Number *(if you know it)*	Date of Filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body
See note (d) — YES

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form.
Do not count copies of the same document

Continuation sheets of this form	0
Description	14
Claim(s)	0
Abstract	0
Drawings(s)	7 + 7 SW

10. If you are also filing any of the following, state how many against each item.

Priority documents	0
Translations of priority documents	0
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	0
Request for preliminary examination and search *(Patents Form 9/77)*	0
Request for substantive examination *(Patents Form 10/77)*	0
Any other documents *(please specify)*	0

11. I/We request the grant of a patent on the basis of this application.

Signature OLSWANG Date 12 December 2003

12. Name and daytime telephone number of person to contact in the United Kingdom Stephen Carter 0207 067 3278

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*
b) *Write your answers in capital letters using black ink or you may type them.*
c) *If there is not enough space for all the relevant details on any part of this form. please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*
d) *If you have answered 'Yes' Patents Form 7/77 will need to be filed.*
e) *Once you have filled in the form you must remember to sign and date it.*
f) *For details of the fee and ways to pay please contact the Patent Office.*

Characterising Body Tissue

Field of the Invention

The present invention relates to methods for the characterisation of body tissue. More specifically, the invention is concerned with the characterisation of body tissue as normal (e.g. healthy) or abnormal (e.g. pathological). The invention has particular, although not necessarily exclusive, applicability to the diagnosis and management of cancer, including breast cancer.

Background

In order to manage suspected or overt breast cancer, tissue is removed from the patient in the form of a biopsy specimen and subjected to expert analysis by a histopathologist. This information leads to the disease management program for that patient. The analysis requires careful preparation of tissue samples that are then analysed by microscopy for prognostic parameters such as tumour size, type and grade. An important parameter in tissue classification is quantifying the constituent components present in the sample. Interpretation of the histology requires expertise that can only be learnt over many years based on a qualitative analysis of the tissue sample, which is a process prone to intra observer variability.

Despite the relative value of histopathological analysis, there remains a degree of imprecision in predicting tumour behaviour in the individual case. Additional techniques have the potential to fine-tune tissue characterisation to a greater degree than that currently used and hence will improve the targeted management of patients.

In existing research in this field, x-ray fluorescence (XRF) techniques have been used to study trace element composition of breast tissue and have shown that breast cancer is accompanied by changes in trace elements and such measurements could contribute to tissue grading [1]. It has also been shown that x-ray diffraction effects can operate as an effective means of distinguishing certain types of tissue [2,3]. Furthermore, it has been shown that

such diffraction effects could be suitably analysed to demonstrate small differences in tissue components and that this analysis could lead to a quantitative characterisation of tissues [4].

Summary of the Invention

It is a general, preferred aim of the present invention to develop quantitative analytical approaches that could add precision to the characterisation of tissues, in particular to distinguish between normal and diseased (e.g. pathological) tissue.

A preferred aim is to add precision to the several subjective components of tissue analysis, most notably those variables 'scored' in breast tumour grading.

In general terms, the invention provides methods for analysing and/or characterising body tissue in which results are obtained by considering a combination of two or more different types of measured tissue properties.

In one aspect, the present invention provides a method analysing body tissue, the method comprising:

obtaining data representing a first measured tissue property of a body tissue sample;

obtaining data representing a second, different tissue property of the tissue sample; and

using the data in combination to provide an analysis of the tissue sample.

In another aspect, the invention provides a method for characterising body tissues as normal or abnormal the method comprising:

obtaining data representing a first measured tissue property of a tissue sample;

obtaining data representing a second, different tissue property of the tissue sample; and

using the data in combination to provide a characterisation of the tissue sample as normal or abnormal.

In either aspect it is preferred that data representing a third measured tissue property is also used in combination with the other data in the analysis or characterisation of the tissue sample.

It is particularly preferred that data representing four or more measured tissue properties is used in combination in the analysis or characterisation of the tissue sample.

Suitable techniques that can be used to obtain the tissue property data include x-ray fluorescence (XRF), energy or angular dispersive x-ray diffraction (EDXRD), Compton scatter densitometry, low angle x-ray scattering and the measurement of linear attenuation (transmission) coefficients.

The tissues properties that are measured may include the composition of the tissue sample, for instance the presence, concentrations and/or proportions of specific elements or organic compounds.

Preferably, in either of the aspects above, the data is used in combination to obtain the desired result by using the data as the input to a predefined calibration model that relates the combined data to one or more tissue characteristics (e.g. normal or abnormal).

In a further aspect, the invention provides a method for creating a tool for the analysis and/or characterisation of body tissue, the method comprising creating a calibration model that relates data representing two or more (preferably three or four or more) measurable tissue properties to one or more tissue characteristics.

The calibration model is preferably produced by using sets of the measured data from tissue samples for which the characteristic(s) (e.g. normal / abnormal) to be determined by the model are already known. These data sets can be used to 'train' the model in a known manner.

Other multivariate analysis techniques may be employed.

Brief Description of the Drawings

Embodiments of the invention are described below by way of example with reference to the accompanying drawings, in which:

Figure 1 is a schematic diagram of EDXRD experimental apparatus employed in the exemplary methods described below according to embodiments of the invention;

Figure 2 is a series of graphs showing average XRF responses;

Figure 3 shows EDXRD scatter profiles for normal and diseased tissue;

Figure 4 shows PLS model predictions for the normal test samples;

Figure 5 shows PLS model predictions for the diseased test samples;

Figure 6 shows predictions of tissue type for the normal test samples; and

Figure 7 shows predictions of tissue type for the disease test samples;

Description of Embodiments

The following describes a study that has been conducted *in vitro* using breast tissue samples. The principles exemplified below are, however, more widely applicable, for example to other tissues and in relation to data obtained from *in vivo* as well as *in vitro* measurements. The principles can usefully be employed in *in vivo* imaging applications.

The exemplary embodiments described below employ x-ray fluorescence (XRF) and energy dispersive x-ray diffraction (EDXRD) techniques to reveal the tissue characteristics. The invention is not, however, limited to these two techniques and other techniques may be used in addition or as alternatives to XRD and EDXRD. Other techniques that might be used include Compton scatter densitometry, low angle x-ray scattering and linear attenuation (transmission) coefficients.

As discussed in more detail below, during the study the concentrations of K, Fe, Cu and Zn were measured in 77 breast tissue samples (38 classified as normal and 39 classified as diseased) using X-Ray Fluorescence (XRF) techniques (in other embodiments, concentrations of other elements or organic compounds might be measured). The coherent scattering profiles were also measured using Energy Dispersive X-Ray Diffraction (EDXRD), from which the proportions of adipose and fibrous tissue in the samples were estimated.

The data from 30 normal samples and 30 diseased samples were used as a training set to construct two calibration models, one using a Partial Least Squares (PLS) regression and one using a Principal Component Analysis (PCA) for a Soft Independent Modelling of Class Analogy (SIMCA) technique. The data from the remaining samples, 8 normal and 9 diseased, were presented to each model and predictions were made of the tissue characteristics.

Three data groups were tested: XRF, EDXRD and a combination of both. The XRF data alone proved to be most unreliable indicator of disease state with both types of analysis. The EDXRD data was an improvement, however with both methods of modelling, the ability to predict the tissue type most accurately was by using a combination of the data.

1. Breast Tissue Samples

The tissue samples measured were obtained from mastectomies, lumpectomies and breast reduction surgery. In regard to the latter, a number of healthy breast tissue samples were obtained. The tissue obtained from mastectomies or lumpectomies, was generally taken from the site of a lesion, classified as invasive ductal carcinoma, and in some cases normal tissue was taken from areas distant to the tumours. In line with the available samples, investigations were made for 38 samples classified as normal and 39 samples classified as diseased. The weight of each of the specimens was of the order of 1 g. Most specimens were of thickness in the range of 2-3 mm. Following excision the samples were kept frozen at – 85^0 C, no processing or sample

preparation taking place between excision and measurement. For both the XRF and the EDXRD measurements the samples were allowed to thaw before being measured in room temperature.

2. *Experimental Procedure*

2.1 XRF

The XRF studies were carried out making use of the European Synchrotron Radiation Facility (ESRF), working on the Bending Magnet beamline BM28[5]. Using a simple arrangement of incident synchrotron radiation, tuned to a photon-energy just above the K-absorption edge of interest, particularly low elemental detection limits are achievable (< 1ppm). The high intensities of XRF available allow for short measurement times, providing for a high sample throughput. For the synchrotron photon beam, the plane of polarisation is the same as that of the electron orbit. Thus for a 90^0 geometry between photons directed on to the sample and the normal to the detector (Si(Li), Gresham Scientific Instruments, Sirius model), the strong linear polarisation of the photon beam provides significant suppression of the scattered photon intensity (fluorescence being unaffected). Given that the detector has lateral extent, the remaining sample-dependent scattered photon (coherent and incoherent) intensity reaching the detector is therefore governed by the solid angle formed between the sample and detector crystal. In addition to providing improvement in the signal to background ratio, control of the scattered radiation intensity allows use of the scattered peak area as a normalisation factor. As tissue is a low Z material, the fact that the detection system cannot resolve the Compton component will not affect the results.

Each element of interest (K, Fe, Cu and Zn) was identified by the photopeak associated with its K_α fluorescence photon emission. In order to seek maximum production of the K_α photons of interest, the samples were irradiated by photons of energy 500 eV above the particular K absorption edge, being an arrangement which also allows for the resolution of the scattered incident peak and the fluorescence response. The exception to this method was for K, where the data were collected using the same incident

photons as that for Fe. In order to quantify the sample concentrations of the elements of interest, calibration curves were constructed for each element.

The calibration standards were aqueous solutions of the elements, the water matrix of the calibration models matching the "wet" nature of the tissue specimens. The following ranges of concentrations were used for the calibrations, as indicative of those expected to be found in tissue:

K: 100, 300 and 1000-4000 ppm in increments of 1000 ppm

Fe: 3-30 ppm in increments of 3 ppm

Cu: 1-10 ppm in increments of 1 ppm

Zn: 2-25 ppm in increments of 2 ppm

The calibration solutions were measured in petri dishes that were sealed with laboratory sealing film (LabSeal, Merck). The tissue specimens were placed on such petri dishes previously filled with purified water and sealed. The specimens were then covered with the same sealing film. The beam size on the specimens was 3 mm x 0.5 mm.

The spectra acquired from the standard solutions were analysed using the software PeakFit (PeakFit™ SPSS Inc, AISN Software Inc) developed for spectroscopy. The spectra were smoothed using a procedure based on deconvolution, leading to the removal of peak broadening effects caused by imperfect resolution of the measuring instruments. The spectra were subsequently fitted using a procedure based on the Levenburg-Marquardt non-linear minimisation algorithm. The fitting process took into account a linear baseline resulting in an estimation of the net total of counts integrated over the width of the photopeak. In order to normalise the fluorescence response, the scattered photopeak area was also calculated. The ratio of fluorescence to scattered photon peak area was then used to derive the relationship between element fluorescence and its concentration.

The tissue samples were irradiated under the same conditions as those used for the standard solutions and spectra were collected for each of the elements of interest. The analysis of the spectra also followed the procedure described for the standard solutions. The least squares fit derived from the calibration data, which relates the ratio of fluorescence to scatter photon peak area and the element concentration was used to quantify the levels of each element in each of the samples. It is acknowledged that only a small area of the sample is irradiated but measurements indicate that the inhomogeneity has been found not to significantly alter the profound differences between healthy and cancerous tissues. No correction has been made for matrix effects has been made in this study. However, the interest here is in the comparison between the levels of healthy and cancerous tissue and as any errors are systematic the comparison is not compromised.

2.2 EDXRD

The EDXRD scatter profile of each of the samples was measured using a technique that utilises the scatter of a polyenergetic photon beam at a fixed scatter angle. This technique has been used for a number of biomedical applications, notably that of estimating bone mineral density [6,7] and more recently for breast tissue analysis [2]. For an overview of applications of X-ray diffraction analysis in crystalline and amorphous body tissue see as an instance Royle *et al* [8].

The experimental set up is shown schematically in figure 1. A tungsten target x-ray tube operating at 70 kV and 15 mA was used, the intrinsic filtration being 1 mm of beryllium. The incident beam is tightly collimated via a slit cut in a dural slab forming a rectangular cross section of dimensions 1 mm x 2 mm. A similar collimation arrangement was set up at a scatter angle of 6° leading to a scattering volume in which the thickness of the sample was enclosed. The samples were mounted in standard 35 mm slide frames, sealed on either side with film 4 microns thick (Ultralene from Glen Spectra Reference Materials). The translator enabled the samples to be moved through the incident beam, in this instance a distance of 3 mm, producing an approximate irradiated volume of 12 mm^3. The scattered photons were detected using an HpGe detector

(EG&G Ortec). The output pulses were analysed using a multi channel analyser (92X Spectrum Master, EG&G Ortec). The measurement time for each sample was 2400 seconds.

3. Results

3.1 XRF

Figure 2 shows the average of all XRF spectral response for the four elements of interest for normal and diseased tissue. Quantitative values of the elemental concentrations were obtained from the ratio of the XRF response peak to the scattered peak (not shown in figures) via the calibration line.

Table 1 summarises these results for all tissue samples, showing the range of concentrations within a sample group, the mean concentrations and the standard deviation. For details about the measured levels of the elements and the comparison between healthy and cancerous tissue see work by Geraki *et al* [1].

Units in PPM	K		Fe		Cu		Zn	
	Normal	Diseased	Normal	Diseased	Normal	Diseased	Normal	Diseased
Range	57-514	114-1120	< D.L.-36.64	< D.L.-48.98	< D.L.-1.53	< D.L.-2.09	< D.L.-7.51	< D.L.-20.19
Mean	163	512	8.82	17.62	0.27	0.88	2.19	6.96
SD	141	336	8.74	14.09	0.22	0.52	1.37	5.31

Table 1: Summary of the XRF results for the elements K, Fe, Cu and Zn.

3.2 EDXRD

Figure 3 shows the averaged diffraction spectra for all normal and all diseased tissue samples. The difference in the composition of the two types of specimens is evident.

The characteristic peak from the adipose tissue can be seen at a momentum transfer value of 1.1 nm^{-1} and the characteristic peak from fibrous tissue is at approximately 1.6 nm^{-1}. These peaks were fitted using the same technique as for the XRF spectra. The evaluated photopeak areas reflecting the presence of adipose and fibrous tissue were then corrected for factors such as the

shape of the x-ray tube spectrum and the difference in the attenuation and scatter properties of the two types of tissue. The corrected relative intensities of the two scatter peaks reflect the relative amounts of the two materials in each specimen.

These results show a strong dependence of the relative amounts of the two materials on the type of specimen. The healthy specimens were predominately made of fat (76 ± 9 %) while the tumour specimens were mainly composed of fibrous tissue (85 ± 4 %).

4. Modelling

The above data were divided into two groups i.e. 30 normal and 30 diseased sample data were used as a training set to produce a calibration model. The data from the remaining samples from each group (8 normal and 9 diseased) were then used as input for the model and the tissue type was predicted.

In the calibration process we use the empirical data (i.e. elemental composition and EDXRD data) and prior knowledge (i.e. quantities known to represent that data) to predict unknown quantitative information from future measurements. In this simple application we are using a multivariate approach i.e. we use many variable measurements X{x(1), x(2)....x(n)} to quantify the target variables Y{y(1), y(2)....y(m)}. In this case the X variables are the data measured above and the Y variables are the categories of tissue i.e. normal or diseased.

In order to predict the later from the former we need to estimate how X relates to Y and an example of this is a regression model of the form y=Xb+c. The unknown parameters b and c are estimated from the calibration data which can then be used to predict future values of y from measured X.

Another method is to use a classification procedure where models are created that represent a particular classification of variable. This is carried out using principal component analysis on the data belonging to a particular classification. Input data are then analysed and compared with the models and a fit to each classification is established.

There are several statistical calibration methods available for creating a model[9] and this work uses a partial least squares (PLS) regression and principal component analysis (PCA), which is used for Soft Independent Modelling of Class Analogy (SIMCA) classification. The Unscrambler [10] is a software package that was used to perform multivariate analysis in this study.

4.1 Partial Least Squares (PLS) regression

Three models were created using the PLS approach. The first used the XRF data alone, the second used the EDXRD data alone and the third used the combined data set. The appropriate data from the test samples (8 normal, 9 diseased) were then presented to the model and predictions of tissue type made.

Figures 4 and 5 show the predictions for the normal samples for all three models and the predictions for the diseased samples respectively.

4.2 Classification models

For each of the data groups (XRF, EDXRD and combined data) principal component analysis models were constructed for the healthy and the cancerous samples. The appropriate data from the test samples were presented to the models and a score was obtained indicating how close each sample came to each of the models.

Figure 6 and 7 show the predictions for each sample in the normal category and diseased category respectively.

5. Discussion

It can be seen that the XRF model predictions using the PLS approach are the most unreliable with the EDXRD and the combined data being similar. However the uncertainty in the predictions becomes significantly smaller when both data sets are combined.

If an acceptable prediction parameter was chosen at 70% certainty and above, the number of reliable and false predictions can be found. Table 2 summarises the data for the PLS model

	XRF		EDXRD		Combined	
	Normal	Diseased	Normal	Diseased	Normal	Diseased
Mean Prediction (%)	72.8	69.8	82.2	98.5	82.6	99.2
Mean Uncertainty (%)	11.2	32.9	20.1	21.6	12.2	14.5
True +ve	3	2	3	6	5	7
False -ve	0	1	0	0	0	0
Undecided	5	6	5	3	3	2

Table 2 : The mean predictions, uncertainty and number of true, false and undecided predictions for each data group and tissue type using PLS.

Similarly, when using the classification technique, the XRF is the most unreliable with an improvement being shown using the EDXRD data. It should be noted that for the normal samples predictions were higher in the normal classification whereas for the diseased samples the wrong classification was made in several instances. The use of the combined data shows a marked improvement in prediction particularly when examining the diseased samples.

As above, choosing a 70% probability cut off limit, the number of accurate predictions can be found. Table 3 summarises the data.

	XRF		EDXRD		Combined	
	Normal	Diseased	Normal	Diseased	Normal	Diseased
Mean Prediction (%)	66.8	53.2	67	59	77.8	77.2
True +ve	3	0	4	3	6	8
False +ve	0	1	0	1	0	0
Undecided	5	8	4	5	2	1

Table 3 : The mean predictions, true and false positives for each data group and tissue type using the classification technique.

The relative inefficiency of the XRF data (compared to the combined XRF and EDXRD results) in successfully and accurately predicting the type of the test specimens is due to the wide spread of concentrations that characterise the

groups of samples, evident by the large associated standard deviations (table 1).

As illustrated by the study described above, embodiments of the present invention can provide improved characterisation of tissue types using a combination of data and an appropriate model. A classification technique has been shown to be particularly successful.

Embodiments of the invention have been described above by way of example. It will be appreciated that various modifications to that which has been specifically described can be made without departing from the invention. For instance, the study described above to exemplify the invention involved the use of only two types of tissue characterising properties. Other embodiments of the invention may use more than two types of tissue characterising properties or alternative characterising properties. Creating a model using samples that are characterised using a variety of useful parameters may develop useful histopathology tools. Provided the different data groups can represent all the parameters one wishes to quantify, the multivariate approach is a promising method for accurate characterisation of samples.

References

1. Geraki K, Farquharson M.J. and Bradley D.A. *Phys. Med. Biol.* 2002 **47** (13), 2327-2339

2. Kidane G., Speller R.D., Royle G.R. and Hanby A.M. *Phys. Med. Biol.* 1999 **44** 1791-1802

3. Polletti M.E., Goncalves O.D. and Mazzaro I. *Phys. Med. Biol.* 2002 **47** 47-63

4. Farquharson M.J., Luggar R.D. and Speller R.D. *Appl. Rad. Isot.* 1998 **48(8)** 1075-1082

5. Brown, S.D., Bouchenoire, L., Bowyer, D., Kervin, J., Laundy, D., Longfield, M.J., Mannix, D., Paul, D.F., Stunault, A., Thompson, P., Cooper, M.J., Lucas, C.A. and Stirling, W.G. *J.Synchrotron Rad.* 2001 **8** 1172-1181.

6. Royle, G.J. and Speller R.D. *Phys. Med. Biol.* 1995 **40** 1487-1498

7. Royle, G.J. and Speller R.D. *Phys. Med. Biol.* 1991 **36** 383-389

8. Royle G.J., Farquharson M.J., Speller R.D. and Kidane G. *Rad. Phys. Chem* 1999 **56** 247-258

9. Martens H. and Naes T. *Multivariate Calibration* Wiley, New York 1994

10. CAMO Computer Aided Modelling A/S 1994 The unscrambler version 5.5. Software for multivariate analysis applying PCA, PCR and PLS including experimental design. Olav Tryggvasonsgt. 24, N-7011 Trondheim, Norway.

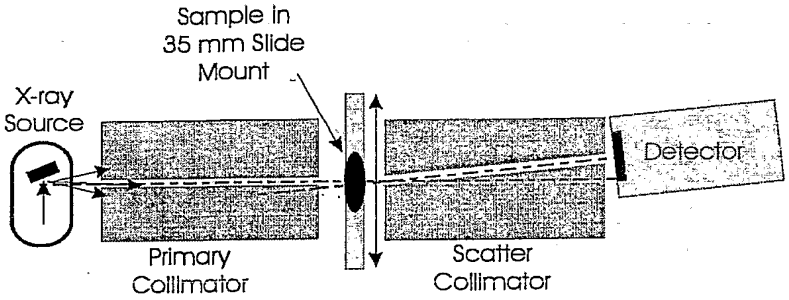
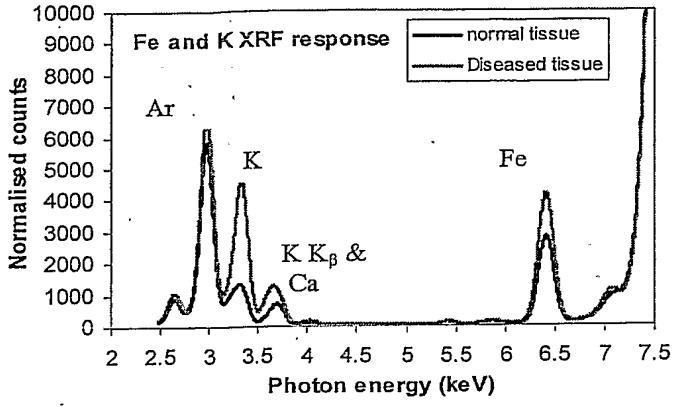
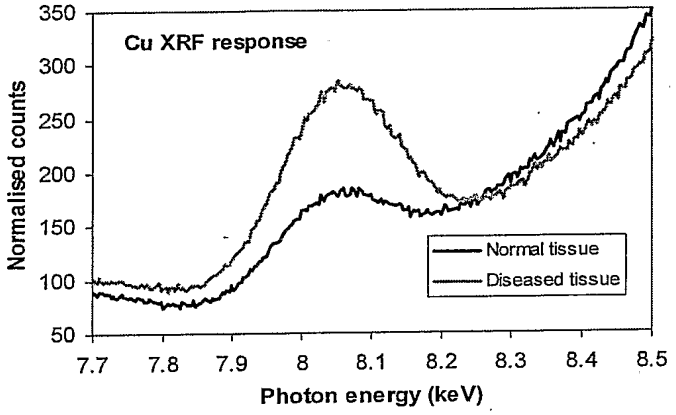


Figure 1

Schematic diagram of the EDXRD experimental apparatus. The primary and scatter slit collimators are 1.0 x 2.0 mm and the scatter angle is 6°.





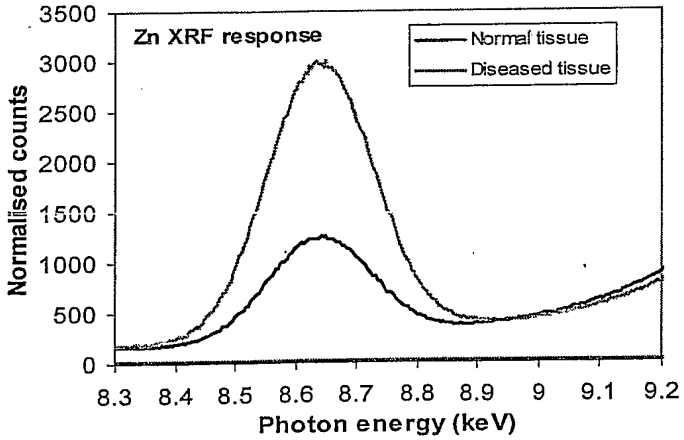


Figure 2

Shows the average XRF response for K, Fe,Cu and Zn. Note also the presence of Ar in air and Ca in tissue.

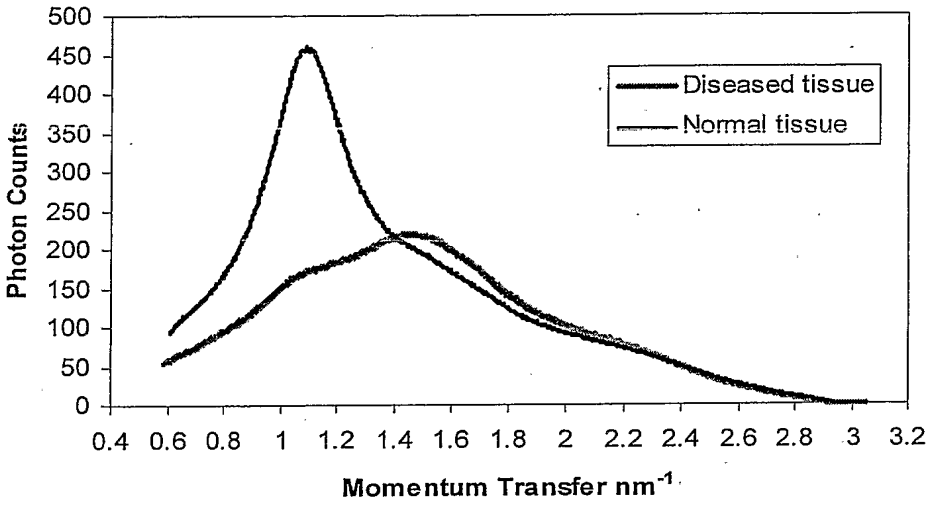


Figure 3

EDXRD scatter profiles for normal and diseased tissue, (average of all samples)

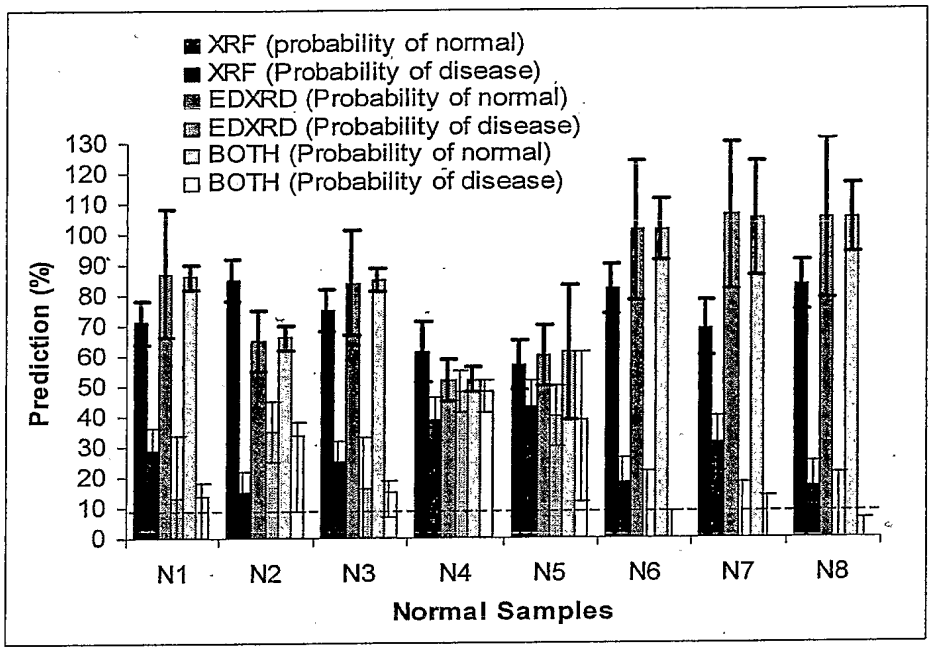


Figure 4

The PLS model predictions for the normal test samples for each data type i.e. XRF, EDXRD and the combination of both. The error bars represent the uncertainty in the predictive ability.

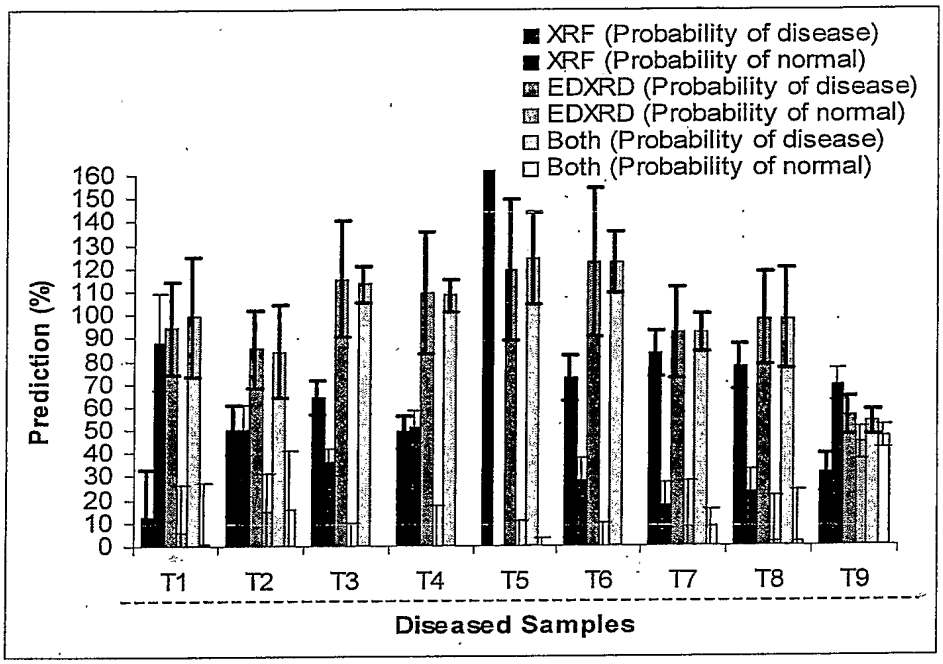
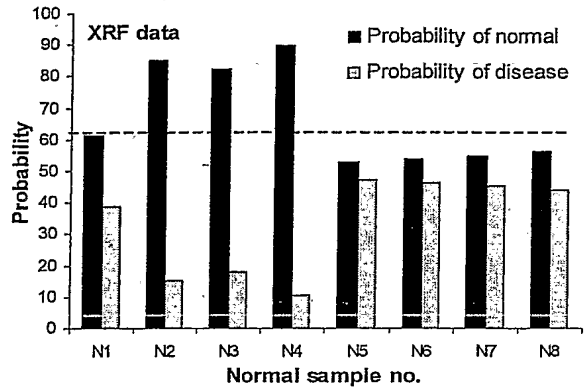
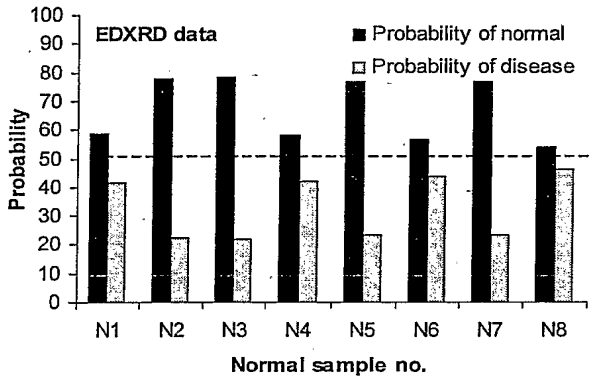


Figure 5

The PLS model predictions for the diseased test samples for each data type i.e. XRF, EDXRD and the combination of both. The error bars represent the uncertainty in the predictive ability.





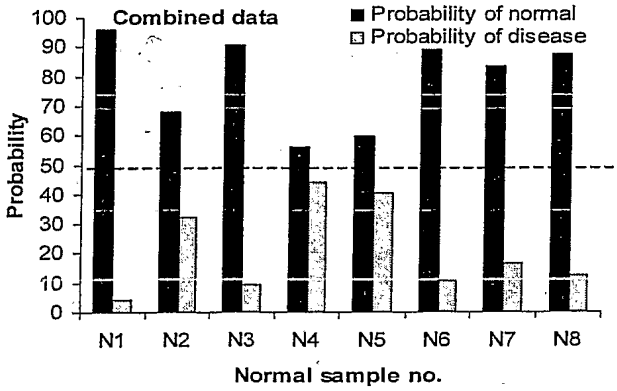


Figure 6

Predictions of tissue type for the normal test samples using the classification technique for the three data groups

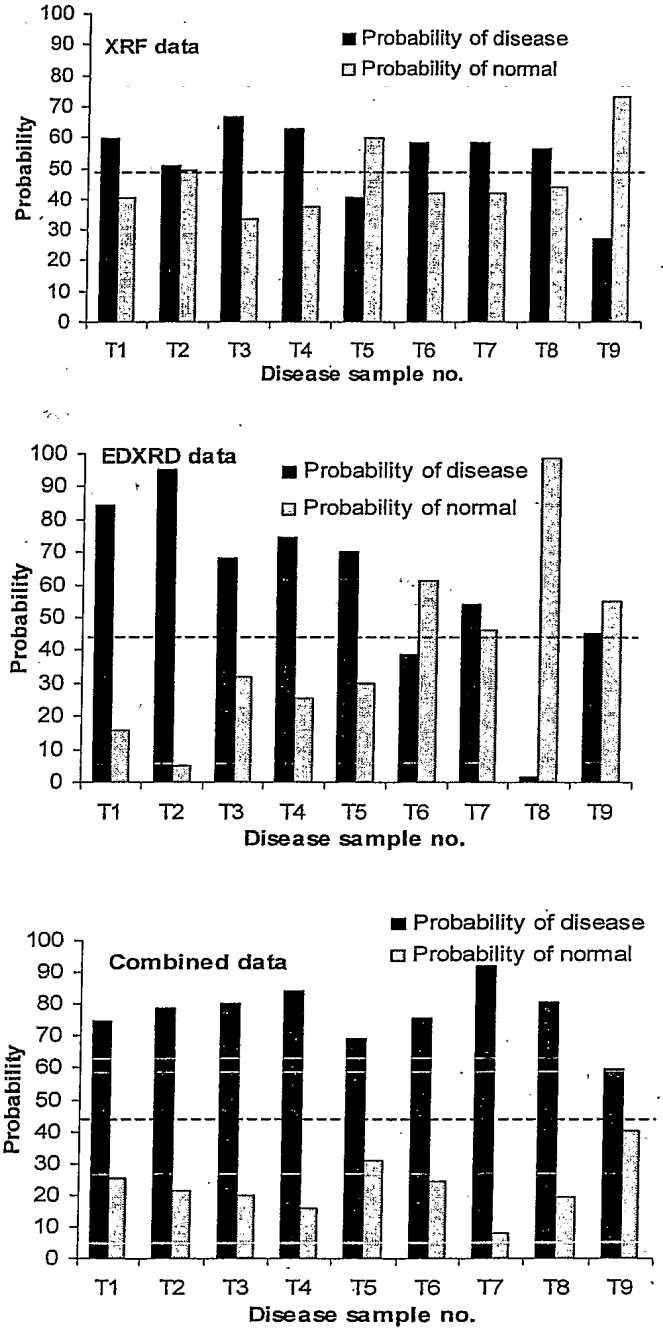


Figure 7

Predictions of tissue type for the disease test samples using the classification technique for the three data groups